

## Microbial aetiology of gastroenteritis, antimicrobial resistance and associated factors among under five children in Addis Ababa, Ethiopia

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### Abstract

**Introduction:** Diarrheal diseases are among the most frequent childhood illnesses and leading causes of preventable death, especially among under five years' children in developing countries. As socioeconomic conditions and living situations are changing, it is important to revisit the bacteriological etiologies and the drug resistance profiles among children with diarrhoea including those under five years old.

**Objective:** To identify enteric pathogens and their antimicrobial susceptibility patterns from under five children in Addis Ababa, Ethiopia.

**Materials and Methods:** A cross-sectional study was conducted from December/2013 – March/ 2014 in selected health facilities of Addis Ababa, Ethiopia. Demographic and clinical data were collected and enteric pathogens were isolated. Antimicrobial susceptibility testing was done following standard bacteriological techniques as recommended by CLIS guideline.

**Results:** Out of 256 children with diarrhoea, enteric bacteria were isolated in 78 (30.5%) participants. The most frequently isolated bacterial pathogen was *E. coli*. There was no single risk factor significantly associated with the microbial aetiology. Sensitivity of the *E.coli* isolates were 30 % to ampicillin; 98 % to ciprofloxacin; 50% to Trimethoprim sulphamethoxazole. The high level (72%) of antimicrobial resistance was detected in *E.coli*, *Salmonella* and *Shigella* isolates. The organisms developed complete resistance to ampicillin and amoxicillin.

**Conclusion:** The first line antimicrobial agents for enteric pathogens are the least susceptible that need culture driven prescription

**Key words:** Diarrhoea, enteric bacterial pathogen, Antimicrobial susceptibility

### Introduction

Diarrheal diseases are among the most frequent childhood illnesses and leading causes of preventable death, especially among under five years' children of developing countries (World Gastroenterology Organization 2008). Diarrhoeal diseases are causes of almost three million deaths in a year for children younger than five years of age (Bryce 2005). Although extensive investigation of diarrhoea has not been reported, the diarrhoea-specific mortality in children younger than five years of age in Africa has been estimated at about 106 per 1000 children (World Bank 2011). The contribution of the various pathogens to diarrhoea may differ substantially between regions depending on local meteorological, geographic, and socio-economic conditions (Hamer et al. 1998). Underlying reasons for the spread of diarrheal diseases are found in poor hygiene and sanitation, limited access to safe drinking water as well as inadequate education of health care providers and recipients (Prakash 2008).

Studies have shown that, in developing countries like Ethiopia, the occurrence of diarrheal disease among under-five children is complex and the relative contribution of each factor varies as a function of

interaction between socioeconomic, environmental, and behavioral variables. Suboptimal infant and young child feeding practices are a major contributor to child morbidity and mortality (Tiruneh 2009). As socioeconomic conditions and living situations are changed, it is important to revisit the bacteriological etiologies and the drug resistant profiles from various study subjects including under five children. Hence, we aimed to identify potential enteric bacterial pathogens and their antimicrobial susceptibility patterns from under five years children in selected health facilities of Addis Ababa, Ethiopia.

### Materials and Methods

**Study area, period, and study design:** Facility based cross sectional study design was conducted from December/2013 to March/2014 in health facilities of Addis Ababa (Selam, Addis Ketema, Semen, and Kolfe Health Centres). Study sites were selected by simple random sampling technique. All under five years children who visited the health facilities for gastroenteritis were included in the study. A total of 256 children were included in the study. A sample size was determined based on single population proportion formula by considering 26.6% prevalence from previous study in Jimma (Tsefahun et al. 2016). Those

children who were taking antibiotics for ten days during data collection and children who are taking vaccination for Rota virus were excluded.

**Specimen Collection and Transportation:** The consented guardian of a child who satisfies the study inclusion criteria was interviewed using structured and pre-validated questionnaire and about five ml of fecal samples was collected using clean, labeled, screw capped and wide-mouthed container. Immediately, cotton tipped swab was immersed in the stool then put in Carry Blair transport medium and transported to Ethiopian Public Health Institute, Clinical Bacteriology and Mycology laboratory in cold-chain system for microbiological investigations.

**Laboratory Investigation:** The swabs were inoculated on an in-house prepared MacConkey agar, Xylose Lysine Deoxycholate (XLD) and Selenite fecal broth. The inoculated plates were incubated at  $35 \pm 2^\circ\text{C}$  incubator aerobically for overnight. Colonial characteristics were examined visually and by conventional biochemical tests (enzyme activity and carbohydrate utilization) were performed for non-lactose fermenter suspected colonies. Bacterial isolates were subjected to antimicrobial susceptibility test using disk diffusion method against Ampicillin (AMP), Cotrimoxazole (SXT), Nalidixic acid (NAL), Ciprofloxacin (CIP) and, drugs selected by CLSI guide line. Disk diffusion test was performed for the isolates on Muller-Hinton agar plates to determine the *in vitro* antimicrobial resistance. Plates were inoculated with standardized inoculums of the bacteria (0.5 McFarland), and overnight incubation at  $37^\circ\text{C}$ . The inhibition zone diameter was measured using caliper in millimeters.

**Quality Control:** Standard operating procedures (SOP) were followed strictly by all laboratory

workers. All culture works were done according to CLSI verification criteria. QC was performed to check the quality of the medium, the potency of the antibiotic and to check manual errors. The quality of each new lot drugs was checked by using ATCC strains of *Escherichia coli* ATCC 25922, *Shigella flexneri* ATCC 12022 and *Salmonella typhimurium* ATCC 13311 strains. Data was accessed by responsible bodies only and were kept up to a year on the principal investigator's personal computer after the study was completed and presented. No third body had access to the hard copies.

## Results

**Sociodemographic characteristics of respondents:** A total of 256 diarrheic stool samples were collected from under five children and investigated for bacterial pathogens. Of all children, 125 were female and 131 were male with a female to male ratio of 1:1.05. The mean age of children was 28.6 months (ranges 2-59 months with standard deviation, SD, of + 15.5). Children with age group of 25- 59 months accounted the highest among all age groups, 132 (51.6 %). Of these 256 children with gastroenteritis (GE), 78 (30.5 %) attended day care and 11 (4.3%) were on exclusive breast feeding.

The mean episode of diarrhea per day was 3.5 (range 1-10, + 1.32 SD) and stayed with GE for an average of 3 days (range 1-10, +1.8 SD). Regarding the sign and symptoms, 134 (52.3 %) had vomiting, 170 (66.4%) had abdominal pain, 51 (19.9%) had tenesmus, and 118 (46.1%) had fever along with diarrhea. The mean episodes of vomiting was 2.2 per day (SD+1.4, range 1-10). The children had an average body temperature of  $37^\circ\text{C}$ , ranges  $35-39^\circ\text{C}$  (+0.55 $^\circ\text{C}$  SD) (Table 1).

**Table 1: Socio-Demographic characteristics and Clinical features of children with acute diarrhea in Addis Ababa, Ethiopia, 2014 (N = 256)**

| Variable            | Category               | Frequency | Percent |
|---------------------|------------------------|-----------|---------|
| Sex                 | Female                 | 125       | 48.8    |
|                     | Male                   | 131       | 51.2    |
| Age Group (Months)  | 0-6                    | 21        | 8.2     |
|                     | 7-12                   | 22        | 8.6     |
|                     | 13-24                  | 81        | 31.6    |
|                     | 25-59                  | 132       | 51.6    |
|                     |                        |           |         |
| Feeding Practice    | Exclusive breast       | 11        | 4.3     |
|                     | Breast plus solid food | 81        | 31.6    |
|                     | Solid food only        | 146       | 57.0    |
|                     | Milk Formula           | 10        | 3.9     |
|                     | Cow Milk               | 8         | 3.1     |
| Vomiting            | Yes                    | 134       | 52.3    |
|                     | No                     | 122       | 47.7    |
| Day care Attendance | Yes                    | 78        | 30.5    |
|                     | No                     | 178       | 69.5    |
| Stool Appearance    | Mucoid                 | 121       | 47.3    |
|                     | Watery                 | 123       | 48      |
|                     | Bloody                 | 12        | 4.7     |

**Socio-Demographic Characteristics of Care Givers:**

Majority, 89.9% of care givers were females with mean age of 29.5 years  $\pm$  6.45 SD ranging from 16 to 57 years and 98 (38.3 %) were in the age group of 28-33 years. Majority of guardians (87.9%) were married, live in private rent home (48.8 %), and had no extra under five children in the household (72.7 %).

Regarding occupation, 100 (39.1 %) of the care givers were employed in private institutions and the mean monthly income of all care givers was 1,533.31 Ethiopian birr (ETB), ranges 0 to 10,000 ETB ( $\pm$  1,411.62 SD). Fifty four percent of guardians gained monthly income with range of 421-1,228 ETB for their family (Table 2).

**Table 2: Socio-demographic characteristics of Diarrheic children Guardians in Addis Ababa, Ethiopia, 2014 (N=256)**

| Variable               | Category         | Frequency | Percent |
|------------------------|------------------|-----------|---------|
| Age Group (Years)      | 16-21            | 21        | 8.2     |
|                        | 22-27            | 78        | 30.5    |
|                        | 28-33            | 98        | 38.3    |
|                        | 34-57            | 59        | 23      |
| Marital Status         | Single           | 6         | 2.3     |
|                        | Married          | 225       | 87.9    |
|                        | Divorced         | 15        | 5.9     |
|                        | Widowed          | 5         | 2.0     |
|                        | Separated        | 5         | 2.0     |
| Type of Housing        | Condominium      | 21        | 8.2     |
|                        | Private villa    | 110       | 43      |
|                        | Private rent     | 125       | 48.8    |
| Educational level      | Illiterate       | 28        | 10.9    |
|                        | Primary School   | 114       | 44.5    |
|                        | Secondary School | 83        | 32.4    |
|                        | Higher Education | 31        | 12.1    |
| Extra <5 Child in home | No               | 186       | 72.7    |
|                        | Yes              | 70        | 27.3    |
| Occupation             | Government       | 26        | 10.16   |
|                        | Private employed | 100       | 39.06   |
|                        | Merchant         | 25        | 9.8     |
|                        | House wife       | 105       | 41      |
| Monthly Income (ETB)   | <421             | 18        | 7       |
|                        | 421-1228         | 138       | 53.9%   |
|                        | > 1,228          | 100       | 39.1%   |
| Latrine                | Available        | 253       | 98.8    |
|                        | Not available    | 3         | 1.2     |

Of all the guardians, 251 (98 %) had piped water source and 96.9 % dispose their waste through waste

collectors, 6 (2.3 %) incinerate and 2 (0.8%) dampened their waste.

**Table 3: Pathogenic Bacteria isolation and Possible risk factor Association among under five children with GE in Addis Ababa, Ethiopia, 2014 (N=256)**

| Variable            | Category         | Pathogenic Bacteria<br>Yes | Growth<br>No | COR (95% C.I)  | P value |
|---------------------|------------------|----------------------------|--------------|----------------|---------|
| Sex                 | Male             | 44                         | 87           | .74 (.43-1.3)  | .27     |
|                     | Female           | 34                         | 91           |                |         |
| Age                 | 0-6              | 11                         | 10           | 1              |         |
|                     | 7-12             | 5                          | 17           |                |         |
|                     | 13-24            | 27                         | 54           |                |         |
|                     | 25-59            | 35                         | 97           |                |         |
| Day-care Attendance | Yes              | 20                         | 58           | 1              |         |
|                     | No               | 58                         | 120          |                |         |
| Feeding Practice    | Breast Only      | 5                          | 6            | 1              |         |
|                     | Breast and Solid | 23                         | 58           | 1.200          | .845    |
|                     | Solid Food Only  | 46                         | 100          | 2.522          | .22     |
|                     | Milk formula     | 0                          | 10           | 2.174          | .29     |
|                     | Cow Milk         | 4                          | 4            |                | .999    |
| Vomiting            | Yes              | 44                         | 90           | .79 (.46-1.35) | .389    |
|                     | No               | 34                         | 88           | 1              |         |
| Abdominal Pain      | Yes              | 52                         | 118          | .98 (.56-1.7)  | .953    |
|                     | No               | 26                         | 60           |                |         |
| Stool Appearance    | Watery           | 33                         | 88           | 1.3 (.376-4.7) | .66     |
|                     | Mucoid           | 41                         | 82           | 1.0 (.28-3.5)  | 1.0     |
|                     | Bloody           | 4                          | 8            | 1              |         |

**Bacterial pathogen gastroenteritis and Association with Risk factors:** Among 256 under five children enrolled, at least one bacterial pathogen was isolated from 78 (30.5%) diarrheic children and *E. coli* was the most frequent isolate contributing 84.6 % among all

bacterial isolates, followed by 12.8% *Shigella* spp. and 2.6% *Salmonella* spp. None of the assumed risk factors were associated with bacterial agents (Table 3 and Figure 1).

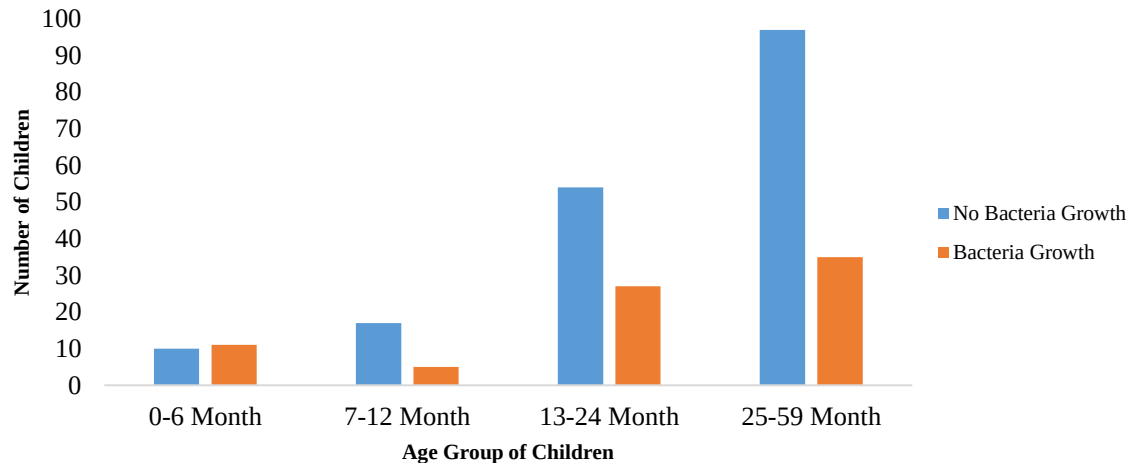


Figure 1: Total bacteria isolates in different age group from diarrheal children in Addis Ababa, Ethiopia, 2014

**Antibiotic susceptibility patterns of bacterial isolates:** Among 78 bacterial isolates tested for antimicrobial susceptibility, 52 (67.5%) of them were resistant to Ampicillin (AMP). Cotrimoxazole (SXT) being

efficacy was only 50%. Generally in this study, non- $\beta$ -lactam antibiotics (Nalidixic Acid (NAL), and Ciprofloxacin (CIP) were found to be more effective ( $\geq 87$ ) against most bacterial isolates.

Table 4: Antibiotic susceptibility pattern of bacterial isolates, Addis Ababa, Ethiopia, 2014

| Isolate | <i>E. coli</i> |           | <i>Shigella</i> spp |           | <i>Salmonella</i> spp. |           |
|---------|----------------|-----------|---------------------|-----------|------------------------|-----------|
|         | S No. (%)      | R No. (%) | S No. (%)           | R No. (%) | S No. (%)              | R No. (%) |
| AMP     | 18 (30)        | 42 (70)   | 5 (50)              | 5 (50)    | 0                      | 2 (100)   |
| NAL     | 46 (86.8)      | 7 (13.2)  | 8 (88.9)            | 1 (11.1)  | 1 (50)                 | 1 (50)    |
| CIP     | 50 (98)        | 1 (2)     | 10 (100)            | 0 (0)     | -                      | 1 (50?)   |
| SXT     | 19 (47.4)      | 21 (52.6) | 7 (70)              | 3 (30)    | 1 (50)                 | 1 (50)    |

S= Susceptible R= Resistance

## Discussion

Annually, an estimated 2.5 billion cases and 1.5 million deaths of diarrhea occur among under five children, being the second killer diseases, contributing one in every five death and the overall incidence has remained relatively stable over the past two decades. More than half of these cases occur in Africa and South Asia where it causes death or other severe outcomes (UNICEF and WHO 2009). The overall prevalence of bacterial diarrhea found in this study was 30.5% (*E. coli*, 25.8%, *Shigella* spp 3.9 % and *Salmonella* spp 0.8 %). This was higher than the finding of a previous study in Hawassa, Ethiopia 22.2%. The possible reason for such difference could be the inclusion of *E. coli* in this study. The prevalence of *Shigella* spp, and *Salmonella* found in this study were much lower than the findings of previous studies, 7 % and 2.5 % for *Shigella* and *Salmonella* respectively in Hawassa, Ethiopia (Getamesay et al. 2014), 15.6 % *Shigella* and 1.6 % *Salmonella* in Gondar, Ethiopia (Kahsay et al. 2011), and higher than 2% *Shigella* and lower than 3% *Salmonella* in Saudi

Arabia (Ayman et al. 2010). The variation in prevalence might be attributed to differences in infrastructures and socioeconomic indices which could have an impact on the transmission of these bacteria.

Our bacterial isolation rate was much higher than the findings of studies conducted in Kenya (Willie et al 2012) and Nepal (Ansari et al. 2012). *E. coli* isolation rate (25.8%) in this study was lower than the finding of a study in Enugu, Nigeria, 41.4% (Korie et al. 2012), but much higher than the finding 2.3 % of a study conducted in Nepal but the burden of *Shigella* spp is in agreement with our report (Ansari et al. 2012). This higher prevalence of *E. coli* relative to other isolates was in agreement with the finding of a study conducted in Kenya (Segecha et al. 2009). The resistance level of *E. coli* isolates for AMP (70%) in this study was higher than the finding of a previous study conducted in Nepal 41.7% but a lower resistance to NAL (13.8%) was found in the current study relative to 33.3% of previous study in Nepal (Ansari et al. 2012).

All 10 *Shigella* isolates obtained in this study were subjected for antimicrobial susceptibility test for AMP and 50% were found resistance, which was lower than the findings of previous studies, 63.6% in Hawassa Ethiopia (Getamesay, 2014) and 100% in Harar, Ethiopia (Ayalu, 2011). *Shigella* isolates were susceptible, 100% for CIP, and 88%, 70% for NAL and SXT respectively. The adjusted CIP susceptibility became 70% because all NAL resistant isolates were reported as CIP resistant. This lower or no resistance level of *Shigella* isolates for these antibiotics were similar with the finding of previous study conducted in Hawassa, Ethiopia (Getamesay et al. 2014) but it varies from the findings of studies in Gondar, Ethiopia where higher resistance level for AMP 80%, SXT 76.7% and CIP 8.3% was found (Kahsay et al. 2011) and in Nepal higher resistance level of 20.8% and 8.3% was found for CIP (Ansari et al. 2012). This could be due to the higher levels of exposure and use of those antibiotics in the community.

The prevalence of bacterial infection in cases of diarrhea among children in other countries varies between 5.3% and 54% (Patel et al. 2008) and antimicrobial resistance by enteric pathogens is of major concern because of indiscriminate use of drugs (Temu et al. 2007). More than 50% of the *E. coli* isolates and 20% *Shigella* isolates were multi drug resistance (MDR), at least resistant for more than two antibiotic disks tested. This resistance level was lower than a previous study conducted in Nepal (58.3% for both isolates) (Ansari et al. 2012). The finding of the current study depicts that CIP is the best effective antibiotics to treat bacterial diarrhea. But, most bacterial isolates had developed resistant for commonly prescribed  $\beta$ -Lactam class of antibiotics like AMP (Mandomando et al. 2007). The high level of resistance of *E. coli* for AMP was due to a wise use of antibiotics in our country. The non  $\beta$ -lactam antibiotics were found to be most effective for bacterial diarrhea treatment. The resistance level of *Shigella spp*s was only high for AMP (50%). The low resistance level of *Shigella spp*s for SXT (30%) is good news for physicians who commonly prescribe it for most HIV/AIDS patients. Although the resistance level of *Salmonella* species found was high for the commonly prescribed  $\beta$ -lactam antibiotics, it is difficult to decide based on this small number of isolates. In this study, there was no significant association between bacterial etiologies and the various risk factors for diarrhea. This might be due to many reasons like inadequate sample size in each variables and the study population could have the same socioeconomic conditions. Moreover, we were unable to isolate bacterial agents like *Campylobacter* species in this study due to lack of culture media

specific to this pathogens and the burden of bacterial agents could be reduced.

## Conclusions and recommendation

The finding of this study indicates that, the most common bacterial pathogen was *E. coli* followed by *Shigella* and *Salmonella* species. The overall antibiotic resistance levels against some commonly prescribed drugs were lower than those reported from other regions in Ethiopia, possibly due to the higher levels of exposure and usage of those antimicrobials in the previous study area. Hence, continuous surveillance of bacterial agents along with antibacterial drug resistance is still recommended to track the real burden and trend of diarrheal diseases.

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